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Full Length Research Paper

Effects of natural essential oil product in normal diet and high-fat-diet fed rats

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Essential oil products are getting popular among the public as health supplements. Most of the products in the market are proclaimed to be effective against metabolic syndrome by improving lipid profile. The aim of this study is to investigate one of the essential oil products in Malaysia on its effect on normal-diet fed rats and high-fat-diet induced obese rats, mainly focusing on the metabolic syndrome and liver toxicity. The product is made of various natural plant oils. Two categories of male Sprague-Dawley rats - the normal diet and high fat diet (HFD) fed categories, were put on a 7-day essential oil treatment. The weight, serum glucose level, lipid profile, liver function test and post-mortem histology analysis were conducted for the evaluation of the product. Mean serum glucose level was insignificantly elevated in the essential oil fed groups of both categories. Lipid profile was insignificantly improved after essential oil treatment in both categories. Insignificant rises on the liver function tests were observed in the treatment group of both categories. The chosen essential oil product insignificantly improved lipid profile in normal diet and high fat diet fed rats, but at the same time it insignificantly deteriorated mean serum glucose level and added burden to the liver.

Key words: Essential oil, metabolic syndrome, liver functions, lipid profile, mean serum glucose, obese rats.

INTRODUCTION

Metabolic syndrome (MetS) or syndrome X refers to an aberrant metabolic function. It is a constellation of disorders which include type 2 diabetes mellitus (T2DM), obesity, hypertension and dyslipidemia (Grundy et al., 2004). This syndrome has been affecting wide regions of the world and is at a rapid increasing pace owing to the bad eating habits and urbanized sedentary lifestyle (Siddiqi et al., 2013). It is also increasingly recognized as the risk factor for the development of cardiovascular disease (CVD). Of all the hallmarks manifested in MetS, dyslipidemia is the condition that is always associated with the manifestation of CVD (Anderson et al., 2013). Dyslipidemia is characterized by abnormal high level of free fatty acid (FFA), triacylglycerol (TAG), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) level, as well as low high-density lipoprotein (HDL) level in the blood (Martin et al., 2008). The elevated plasma FFA is the main defect leading to the development of dyslipidemia and insulin resistance, which is also the other prominent hallmark of MetS (Lim et al., 2009). Apart from causing the onset of MetS, bad eating habit causes non-alcoholic fatty liver disease (NAFLD) as well. The study conducted by Gaggini et al. (2013) demonstrated the connection between NAFLD, dyslipidemia, insulin resistance, atherosclerosis and MetS. NAFLD is a chronic condition ranging from benign steatosis, to more serious liver damage such as lobular inflammation, hepatocyte ballooning, fibrosis and cirrhosis. Besides, NAFLD is often characterized by excess liver fat, and the ascending prevalence of NAFLD is related to the increasing prevalence of acquired obesity. There are approximately 20 to 80% of dyslipidemic cases associated with NAFLD, which means patients diagnosed with dyslipidemia are not only on high risk to diabetes and deadly CVD, but also NAFLD (Gaggini et al., 2013).

Since dyslipidemia is associated with many significant diseases and the improvement of it helps improve

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Table 1. Ingredients of 300 µL of essential oil in each 600 mg soft gel.

Ingredient	Amount (mg)
Pericarpium *Oryza sativa* oil (rice skin oil)	60
Semen *Borago officinalis* oil (Borage seed oil)	60
Semen *Helianthus annus* oil (sunflower oil)	60
Semen *Perilla frutescens* oil (Perilla seed oil)	40
Semen *Camellia oleifera* oil (tea seed oil)	30
Semen *Olea europaea* oil (olive oil)	30
Semen *Linum usitatissimum* oil (flax seed oil)	20
Semen *Persea americana* oil (avocado seed oil)	40
Cucurbita pepo oil (pumpkin seed oil)	60

underlying medical condition (Lavie et al., 2011), the treatment of dyslipidemia has therefore been targeted by many medical personals worldwide. Up to date, statins remain the first-line therapy for treating dyslipidemia over many other drugs owing to its effectiveness in lowering LDL cholesterol and TAG level while increasing HDL level in MetS and CVD (Siddiqi et al., 2013; Ling and Hilleman, 2012). However, many cases of side effects were reported in spite of its benefits. For instances, low dosage of statins fail to exert its effectiveness while increased dosage of statins increase the risk of getting myopathy and hepatotoxicity (Siddiqi et al., 2013). On top of that, most of the medications for dyslipidemia are reported with side effects (Medscape, 2015) that this phenomenon has driven the treatment direction to natural supplement with similar activity.

Essential oils are one of the famous alternative treatments that the public opts for various illnesses (Rozga et al., 2013). It is a mixture of volatile compounds produced as secondary metabolites in plants which are isolated by steaming and distillation from either a whole plant or parts of the aromatic and medicinal plant (Baser and Buchbauer, 2009; Charzifragkou et al., 2013; Lejonklev et al., 2013). The presence of essential oils in plants serves as protective agents against the invaders such as microorganism (anti-microbial property), insects and herbivores that feed on the plant (Chatzifragkou et al., 2013). It has been well reported that essential oils possess various biological activities like antimicrobial, antiparasitic and antioxidant properties and have been widely employed in industrial applications such as pharmaceutical compounds, food preservatives and as mixtures in cosmetics and perfumes (Chatzifragkou et al., 2013). Apart from that, some products of essential oil in the market claim to possess lipid-lowering effect. However, little did the public know that some of the essential oil products in the market have never been subjected to clinical trials and the beneficial effects reported are, most of the time, rested heavily on anecdotal proof (Rozga et al., 2013). Hence, in this study, a product of essential oil supplements in the market was evaluated for its dyslipidemia activity in Sprague Dawley rats. The product chosen is in capsule form with 300 µL of mixtures of various natural plant oils, the detail of which will be discussed subsequently.

METHODS

Animals and treatment

The approval of using animal was sought from the Taylor's University Animal Ethics Committee prior to the commencement of the project. The approval code is TUL 2013-002. Sprague Dawley rats of weight between 250-300 g were supplied by Monash University Animal Facility (Malaysia) and were bred in the animal holding of the Taylor's University Lakeside Campus (Malaysia). The rats were individually kept in polypropylene cages at a controlled temperature of 25°C±1 and humidity of 55±10% with a 12-h light and dark cycle (lights on at 0815 h and lights out at 2013 h).

In vivo assay

The rats were randomly distributed into 4 groups of five rats each – Groups A, B, C and D. Groups A and B rats were fed normal diet with daily amount of 25 g standard rat chow (Goldcoin, Malaysia) and tap water, while Groups C and D were fed with daily amount of 25 g high fat diet (HFD) and tap water for two weeks prior to the treatment. Each group of rats was then subjected to respective treatment through gavage for 7 days, with the same diet going on. Groups A and C rats were put on 100 µL saline, while Groups B and D rats were on the 100 µL essential oil treatment. In summary, the categories of rats were as listed below:

Group A – normal diet + 100 µL saline
Group B – normal diet + 100 µL essential oil
Group C – HFD + 100 µL saline
Group D – HFD + 100 µL essential oil.

The essential oil fed to Groups B and D is a commercialized product and it is enclosed in soft gel. The ingredients of 300 µL of essential oil in each 600 mg soft gel are stated in Table 1.

The high fat diet fed to Groups C and D were prepared by mixing powdered rat chow (Goldcoin, Malaysia) with vegetable shortening (Nona, Malaysia) that contributes 60% of the total calories in the ratio of 2:3 (Eu et al., 2013). The food and water consumption were maintained and checked on daily basis throughout the whole experimental period. The weight of each rat was measured on four-day basis for observation.

Sample collection

Upon the completion of the treatment period of 7 days,

rats were put to 12 h fast prior to humane sacrifice under the anesthesia of combined ketamine – Narketan®-10 (Vetoquinol, UK) (75 mg/kg) and ilium xylazil-20 (Troy Laboratories, AUS) (5 mg/kg) via the intraperitoneal injection. Blood was drawn from the cardiac ventricle through the apex of the heart and was allowed to clot at room temperature (25°C) for 30 min before the 10 min centrifugation at 4°C, 12,000 × g. The serum was collected and immediately stored in -80°C for future analysis. However, the left and right lobes of the liver were collected for histological analysis.

Serum biochemical analysis

Serum glucose level was determined using Trinder's Glucose Oxidase method. The procedures were carried out using an automated chemical analyzer LWC100 (Landwind, Hungrary) following instructions given by the Glucose Oxidase kit (Stanbio, USA).

Serum lipid profile of total cholesterol, total triacylglycerol (TAG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined using Stanbio Cholesterol LiquiColor® Test (Stanbio, USA), Stanbio TriacylglycerolLiquiColor® Test (Stanbio, USA), Stanbio Cholesterol HDL Direct LiquiColor® Test (Stanbio, USA) and Stanbio Cholesterol LDL Direct LiquiColor® Test (Stanbio, USA), respectively. All the procedures were performed by automated chemical analyzer LWC100 (Landwind, Hungrary). Serum free fatty acid concentration was determined using Randox FA115 Non-Esterified Fatty Acids kit (Randox, UK). Serum concentration of very low density lipoprotein (VLDL) was calculated based on the formula - TAG concentration / 5 (Nabi et al., 2013).

Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) were used to assess the liver functions of rats. Stanbio AST/SGOT Liqui-UV® Test (Stanbio, USA), ALT/SGPT Liqui-UV® (Stanbio, USA) and Alkaline Phosphatase LiquiColor® Test (Stanbio, USA) were used to estimate SGOT, SGPT and ALP respectively.

Histological analysis

Livers collected were fixed in 10% neutral-buffered formalin until hardened. Then each of the tissues was subjected to impregnation, infiltration, embedding and sectioning process (Chia et al., 2012). Tissue sections of 5 µm thickness were stained with Hematoxylin and Eosin (H&E) stain and were viewed under a light microscope (Nikon Eclipse 55i) and photographed with the attached camera (Nikon DS-Fi2).

Statistical analysis

Data collected were analyzed using Statistical Package for the Social Sciences (SPSS) Version 16.0 (SPSS Inc., USA) and results were expressed as means ± standard error of means (SEM). Paired-samples *t-test* was used for the analysis of weight before and after the treatment. Independent samples *t-test* was used for the analysis of the remaining data with the pairing comparison of Groups A versus B and Groups C versus D. All data were parametric distributed. A p value of ≤0.05 was considered significant.

RESULTS

The results of the experiment were divided into four main parts, which are (i) the comparison of mean weight of rats (±SEM) in each group before and after treatment; (ii) the mean serum profile of rats in each group (±SEM) with parameters namely serum glucose, total cholesterol, TAG, FFA, HDL, LDL, and VLDL levels; (iii) the mean score (±SEM) of selected liver function tests – SGOT, SGPT and SLP; and (iv) histological images of heart and liver tissues of rats in each group. Other than (i) as mentioned, all parts were analyzed by comparing Groups A to B (the normal-diet fed category, Group A is the control and Group B is the essential-oil treated group) and Groups C to D (the high-fat-diet fed category, Group C is the control and Group D is the essential-oil treated group).

Before treatment, the mean weight difference between the HFD category and the normal diet category was analyzed to confirm the condition. There was a significant rise (p ≤0.05) in the mean weight of the HFD category (Groups C and D) as compared to the normal diet category (Groups A and B). Comparison was also made within each group and insignificant change in the mean weight was observed in all groups (p >0.05). As shown in Figure 1, rats in Group D (group fed with high fat diet + essential oil) had the biggest weight change with a percentage of 9.07% (p >0.05), followed by rats in Group C (group fed with high fat diet + saline) with 7.28% (p >0.05).

The mean serum glucose of rats in Group B was 28.02% (p >0.05) higher as compared to rats in Group A. The same trend was observed in Groups C and D, where 9.82% (p > 0.05) higher in the mean serum glucose was observed in Group D rats as compared to rats of Group C. The result is shown in Figure 2.

The comparison of the lipid parameters between Groups A and B, Groups C and D is shown in Figures 3 and 4. The difference of all the chosen parameters in lipid profile between Groups C and D in the high-fat-diet fed category was insignificant (p >0.05). In the normal-diet-fed category, there was an insignificant rise (p >0.05) in the mean serum total cholesterol, mean serum LDL, mean serum HDL and mean serum FFA in Group B as compared to Group A. The mean serum TAG level of the essential-oil treated Group B was significantly lower by 15.46% than that of Group A (p ≤0.05). Apart from that, the mean serum VLDL of Group B rats was also significantly lower as compared to Group A (p ≤0.05), with a 15.46% difference.

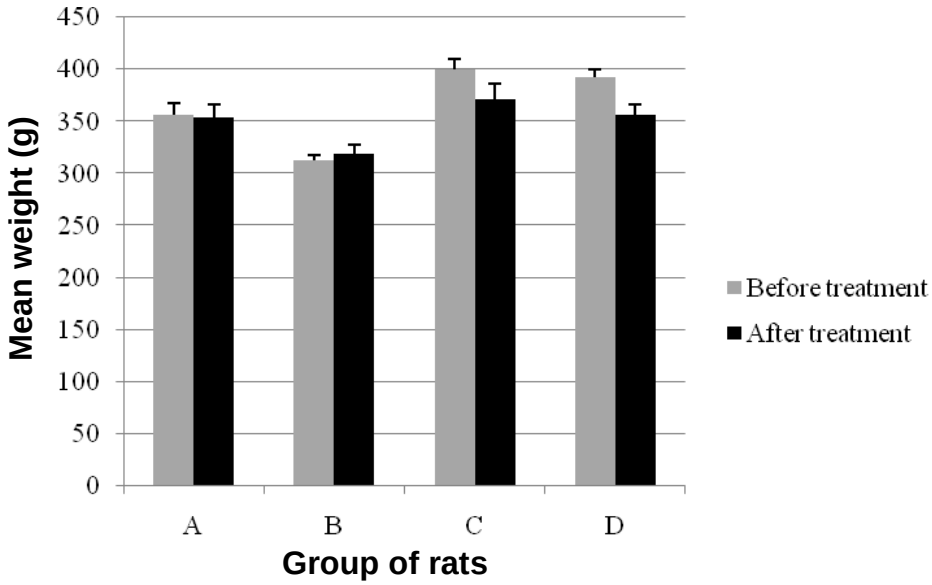

Figure 1. Mean weight (as g) of rats in Groups A, B, C and D before and after treatment.

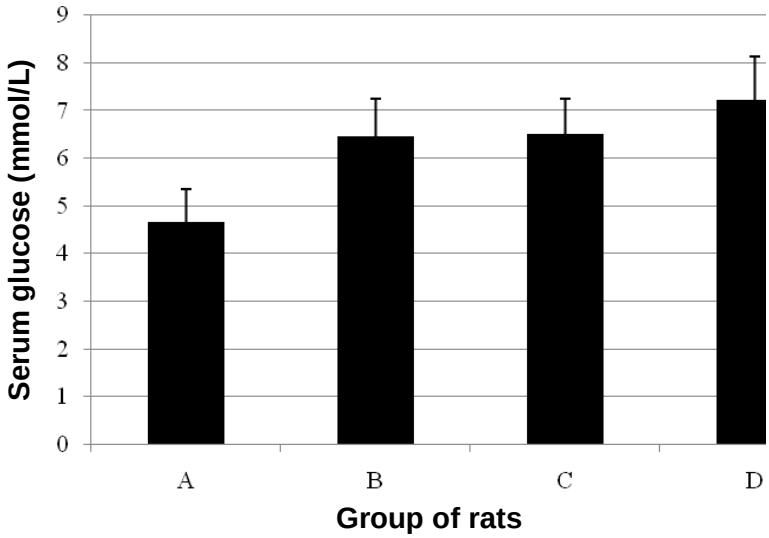

Figure 2. Mean serum glucose (as mmol/L) of rats in Group A, B, C and D.

As for the high-fat-diet fed category, Groups C and D, insignificant reduction in the mean serum total cholesterol, TAG, VLDL and FFA was observed in essential-oil treated Group D rats in comparison to Group C rats. The reduction for the mentioned parameters was 13.40%, 2.07%, 0.69% and 1.16% respectively (p >0.05). The mean serum LDL and HDL levels of Group D rats were insignificantly higher (p>0.05) as compared to Group C rats; the rises were 3.96% and 6.38% each.

The results of liver function test of all groups were displayed in Figure 5. In the normal-diet fed groups, the mean serum AST level of Group B rats was insignificantly higher by 29.40% (p >0.05) as compared to rats of Group A. Similar trend was observed in the high-fat-diet fed groups where Group D rats showed 16.28% higher mean serum AST reading compared to the control Group C rats (p >0.05). As for mean serum ALT level, the essential-oil-treated rats (Groups B and D) in both normal-diet and high-fat-diet fed categories showed insignificant higher value (p >0.05) than the control groups, by 5.31% and 15.42% respectively. In comparison to the control groups, both the essential-oil treated groups given normal diet

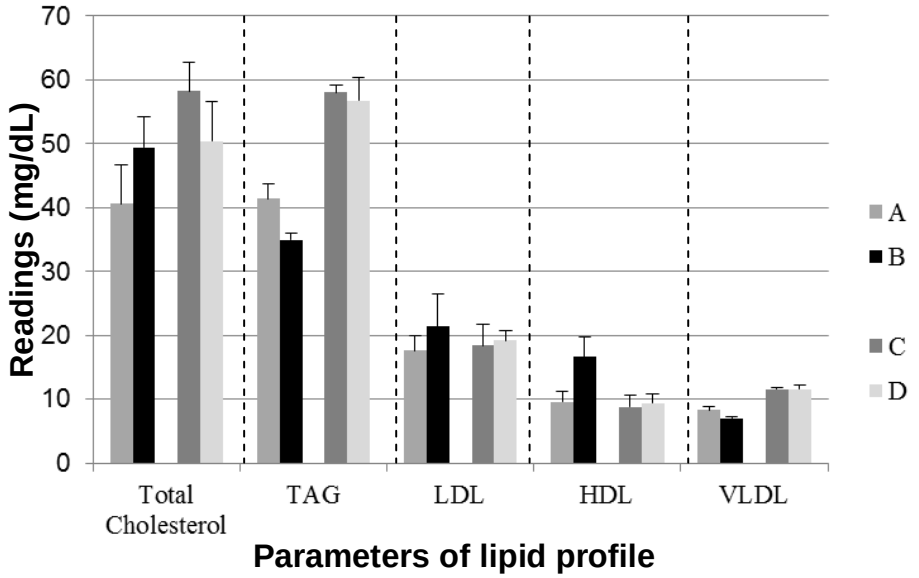

Figure 3. Lipid profiles of various parameters (as mg/dL) of rats in Groups A, B, C and D.

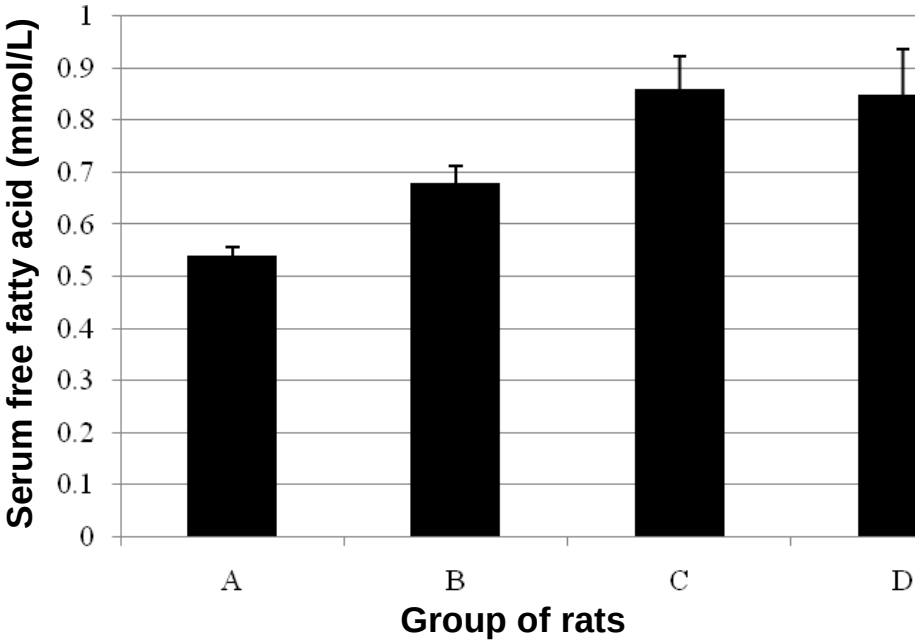

Figure 4. Mean serum free fatty acid (as mmol/L) in rats of Group A, B, C and D.

and high fat diet showed higher level (p >0.05) of mean serum ALP, in which Group B was shown to be 19.32% higher than Group A; while Group D was 17.09% higher than Group C.

The H&E sections from the liver were displayed in Figure 6. Liver sections from the rats of normal-diet fed category (Groups A and B) displayed normal architecture of liver morphology comprising cords of hepatocytes radiating from the central vein. There was no evidence of fatty change in either of the sections.

Liver sections from Group C displayed areas of extensive adipose change. Both microvesicular and macrovesicular types of steatosis were observed in the liver. As for the essential-oil treated Group D, moderate steatosis was observed with predominant microvesicular type and minimal macrovesicular type being observed in

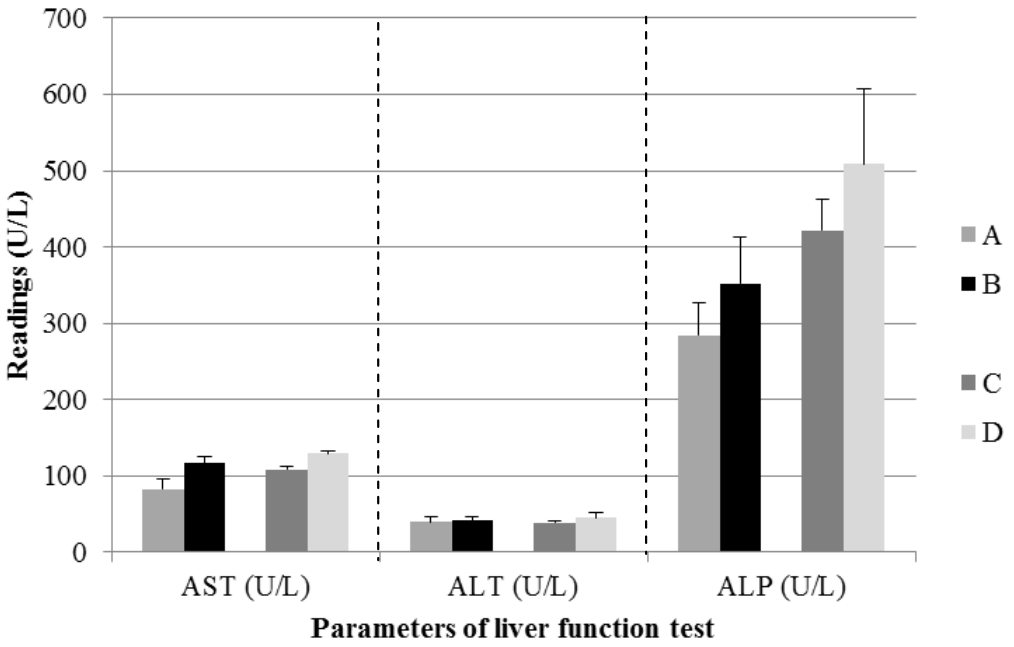

Figure 5. The chosen parameters of liver function test (as U/L) of rats in Groups A, B, C and

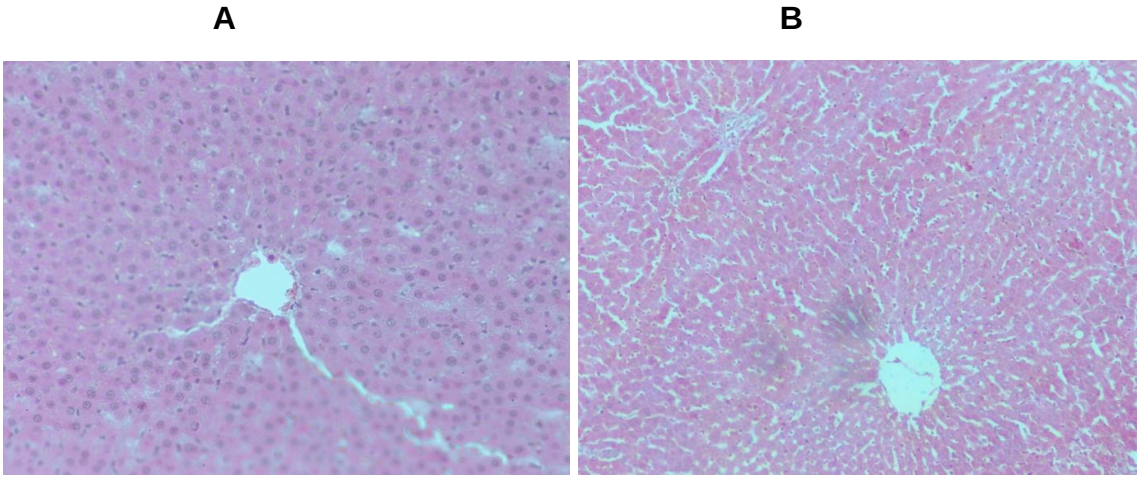

Figure 6. Liver sections stained with H&E stain from normal-diet fed groups, with Group A on the left (A) and Group B on the right (B). Total magnification is 200×.

some areas, showing slight improvement in fatty change as compared to Group C non-treated rats. Images are shown in Figure 7.

DISCUSSION

Consuming dietary supplements has become a popular option to most of the people in the recent years for healthy lifestyle and as an intervention for various health issues (Rozga et al., 2013). Rozga et al. (2013) stated that over 50% of adults are using dietary supplements for self-treatment. The increasing usage of dietary supplement is uncertain whether due to an increased need for self-treatment owing to the rising medical costs, or it is just merely the marketing gimmick of companies for the sales of their products. A serious concern had been raised in the same study that the manufactures of dietary supplement, although, is regulated by the FDA, but manufacturers do not have to prove the safety or efficacy before a dietary supplement is marketed or sold.

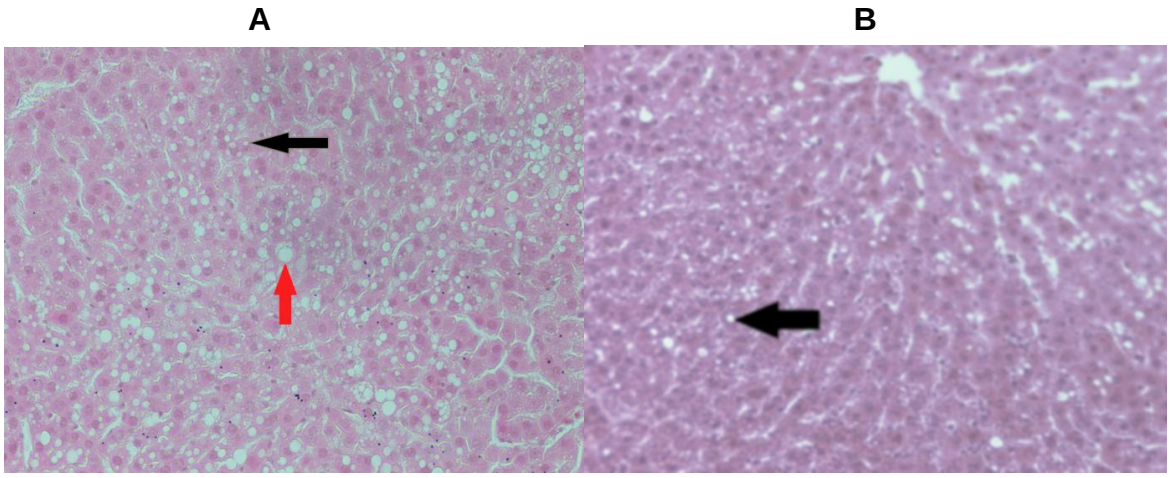

Figure 7. Liver sections stained with H&E stain from the high-fat-diet fed groups, with Group C on the left (A) and Group D on the right (B). Grey arrow indicates macrovesicular steatosis, while black arrows indicate microvesicular steatosis. Total magnification is 200×.

Therefore, it is sad to say that there are many untested products in the market which has been widely consumed by the innocent citizens. In this study, we aim to evaluate the properties of a marketed essential oil supplement to check for its probably self-claimed ability to improve dyslipidemia, particularly high cholesterol. Apart from that, selected liver function tests were also performed in this study to evaluate the possible side effects of the product which is not included in the user instruction of the product. This study was carried out on two categories of rats, which are the normal diet fed category and the high-fat diet fed category.

The mean weight of rats in the HFD category (Groups C and D) were significantly higher than the normal diet category (Groups A and B). It has been well documented that high fat diet causes obesity in both human and rat models (Hariri and Thibault, 2010). As reported in the review by Hariri and Thibault (2010), the fat energy requirement for rat is approximated at 5%. Obesity can be induced in rats by giving diets containing fat as low as 13% of total energy to 85% of energy. In our case, the high fat diet given to both Groups C and D was constituted with 60% of fat energy, thus, causing the significant rise in the body weight as compared to the normal diet-fed groups. Besides, high fat diet was proven to lower satiety level (Hariri and Thibault, 2010), in that it also played a role in causing overconsumption of food in our HFD-fed Groups C and D rats, causing the significant weight gain. It was confirmed that the induction of obesity in the HFD category was successful.

Seven days treatment started after the two-week induction period. The effect of essential oil in weight change was insignificant in the normal diet category. Insignificant weight loss ($p > 0.05$) was observed in the HFD category in both Groups C and D. Hence, it cannot be postulated that the weight loss in Group D was due to the effect of essential oil as weight loss was also observed in the control – Group C. According to Fernando et al. (2013), improper practice of gavage might stress the animals and alter the metabolic effect that might further alter the weight change. This possibility was ruled out as the same researcher was handling both the categories of normal diet groups and HFD groups at the same time; if it were due to the mentioned possibility, weight loss should have been observed in both groups in the normal diet category as well. The reason for the weight loss was uncertain in the HFD category but it was postulated that it did not come from the effect of essential oil treatment as weight loss was not observed in the Group B rats of normal diet fed category.

There was a trend in the glucose profile of rats in both the normal diet and HFD categories, where the rats fed with essential oil were shown to have an insignificant higher ($p > 0.05$) blood glucose level than the controls in each category, showing a rise in the blood glucose after the essential oil treatment. Apart from that, rats in the HFD category was also shown to have insignificant higher ($p > 0.05$) mean blood glucose in comparison to the normal diet category, by comparing the individual group. High levels of fat in the diet or high levels of free fatty acid (FFA) interfered the on and off of two transcription factors known as FOXA2 and HNF1A. These two transcription factors are responsible for the synthesis of GnT-4a glycosyltransferase (Ohtsubo et al., 2011). GnT-4a glycosyltransferase was found lacking in mice reported with type 2 diabetes and it functions to generate the core β1-4GlcNAc linkage among the N-glycan structures and promotes the surfacing of glucose transporter-2 (Glut-2) glycoprotein needed for glucose uptake and glucose-stimulated insulin secretion in beta

cell. It was postulated that high fat diet or high levels of FFA was found to down-regulate the expression of both FOXA2 and HNF1A that further depletes GnT-4a glycosyltransferase for glucose uptake (Ohtsubo et al., 2011), thus, explaining the glucose profile observed in our rats. The essential oil chosen is rich in monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) (McKevith, 2005). It is presumed that these excessive fatty acids had a role in the GnT-4a glycosyltransferase that caused the elevated mean blood glucose in the treatment group. This is also supported by our results of insignificant higher mean FFA levels in the essential treatment group in the normal diet and HFD categories. PUFA and MUFA had been well documented to be protective against type 2 diabetes (Kaseb et al., 2013), but there was a study which observed that type II diabetic patients were found to have aggravated blood glucose level and insulin resistance after an intake of high-dose long-chain n-3 PUFAs (5-8 g/d). The possible explanation is that n-3 PUFA contains alpha-linoleic acid (ALA). Excessive ALA induces fatty acid β-oxidation that increases acetyl-CoA accumulation which promotes citric acid accumulation through the inhibition of pyruvate dehydrogenase activity. Accumulated citric acid blocks glucose oxidation in the initial stage of citric acid cycle that signals the decreased glucose transportation by GLUT4 (Zhang et al., 2014). Flax seed oil is rich in n-3 PUFA and therefore, the increase of mean serum glucose level observed in the essential oil treated rats may be due to the stated reason.

There was an insignificant improvement in the lipid profile of rats fed with the essential oil in the HFD category (Group D) in comparison to the control, except for the mean serum LDL. However, different trends were observed in the normal diet category. Significant improvement in mean serum TAG, HDL and VLDL was observed in Group B rats, while insignificant rise on the total cholesterol and LDL was observed in the same group of rats. The reason to the increased total cholesterol in Group B rats was due to the rise in HDL and LDL of the rats, as total cholesterol comprises HDL, LDL and VLDL (McConnel, 2007). There are contradicting reports regarding n-3 PUFA on the LDL levels. Some reports demonstrated the efficacy of n-3 PUFA in reducing LDL level; several reports indicate that n-3 PUFA does not affect LDL level, while some reported that LDL level increased after n-3 PUFA consumption (Carpentier et al., 2006; Olalla et al., 2009; Ill et al., 2013). In our case, the mean serum LDL of essential oil treated rats of both normal diet and HFD categories fit into the latter observation, which is the consumption of essential oil increases in LDL level. The increase of the LDL level in rats given essential oil treatment does not seem to be associated with increased cardiovascular risk as there was an increase in the mean serum HDL level in these rats, which this phenomenon compensated for the rise of LDL level. The rise in HDL level in these rats could be due to the TAG lowering effect of the essential oil that affects the cholesteryl ester transfer protein-mediated exchanges (Carpentier et al., 2006).

Rich MUFA has been reported in *Helianthus annus* (sunflower), *Olea europaea* (olive), and *Persea americana* (avocado seed), while PUFA has been reported in *Helianthus annus* (sunflower), *Olea europaea* (olive), *Linum usitassimum* (flax seed) and *Cucurbita pepo* (pumpkin seed) (McKevith, 2005; Ill et al., 2013). Both MUFA and PUFA are well known for its effectiveness against dyslipidemia (Houstan, 2012). Besides, *Olea europaea*'s phenolic compounds decrease LDL and TAG, while increasing HDL (Cicerale et al., 2010); and *Oryza sativa* (rice skin) oil contain various bioactive compounds such as γ-oryzanol, coenzyme Q10, tocopherol, tocotrienols, polyphenol and some phenolic acids such as ferulic acid, p-coumaric acid and diferulate that showed positive activity in lowering serum total cholesterol, LDL and TAG concentration (Kongkachuichai et al., 2012). Studies showed that γ-tocotrienol helps increase LDL-receptor and HMG-CoA reductase mRNA expression that aid in serum TAG and LDL reduction. γ-oryzanol and ferulic acids are effective against hypercholesterolemia and lipid profile by increasing fecal cholesterol and TAG excretion, which is shown in the hypercholesterolemic mice after 7 weeks of *Oryza sativa* indica oil treatment (Kongkachuichai et al., 2012). The improvement of the lipid profile in essential oil fed rats in Group D could also be due to all these compounds in the essential oil. These results were also aligned with the histological liver images of the rats in Group D, as the condition of steatosis in Group D rats was better than the non-treated rats under the same category.

Liver function tests were performed to assess the possible adverse effects of the essential oil. ALT, AST and ALP were chosen as these three are commonly used for the liver function measurement (Kaplowitz and Deleve, 2013). These enzymes are markers for intact liver function and are normally elevated in acute hepatotoxicity or mild hepatocellular injury but are reduced in the prolonged intoxication due to the severe damage caused to the liver (Abdel-Moneim et al., 2011). The volume of essential oil administered to the rats was translated from the dosage stated in the consumption manual of the essential oil by employing formula adopted from the study of Reagan-Shaw et al. (2007). Generally, the mean serum AST, ALT and ALP were insignificantly higher in the rats given essential oil (Groups B and D), which indicates the intake of the essential oil had added burden to the liver of the rats. The study of Abdel-Moneim et al. (2011) showed the hepatoprotective activity of flaxseed oil in rats given lead acetate-mediated hepatic oxidative stress, while on the other hand, PUFA is also a proven hepatoprotective agent (Chaven et al., 2013). These suggest the possibility of unreported additives in the essential oil product and the prolonged consumption might lead to the development of NAFLD.

Overall, the findings of this study had demonstrated the insignificant beneficial effects of the essential oil product in improving dyslipidemia as proclaimed by the manufacturer. However, minimal liver injury was demonstrated in this study as well, which was not revealed by the manufacturer. Long-term consumption of this product could aggravate liver injury and the hidden side effects could outweigh the beneficial effects of this product in a long run. This study does not represent the entire natural supplement and dietary supplement products in the market, but it highlighted the possible unreported adverse effects of consuming these products in a long run.

Conclusion

In conclusion, the essential oil product showed no significant activity on the weight changes in rats under both normal diet and HFD categories. The mean serum glucose of rats given 7-days essential oil treatment was seen to insignificantly increase in both the normal diet and HFD categories. However, the product showed significant improvement on the mean serum TAG and VLDL levels, as well as insignificant improvement on the mean serum HDL level of rats under normal diet category. Insignificant improvement on the mean serum total cholesterol, TAG, HDL and VLDL levels were also observed in rats given 7-days essential oil treatment under the HFD category. Improvement of liver steatosis was also observed in the liver images of the particular group as well. On the downside, insignificant raise of mean serum AST, ALT and ALP were observed in rats given essential oil treatment in both the normal diet and HFD categories which suggested that the essential oil product had added burden to the rats' liver.

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